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Efficiency of *Hericium erinaceus* Production with Spent Coffee Ground Augmentation

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Abstract

Hericium erinaceus (lion's mane) is a commonly cultivated fungi that has shown promise in recent studies investigating its medicinal effects. Studies have shown the ideal substrate for cultivation of *H. erincaceus* is a mixture of hardwood sawdust and wheat bran. Additionally, certain food waste products have been shown to supplement the substrate mixture effectively. No studies have investigated how the addition of spent coffee grounds (SPG) to the substrate mixture on *H. erincaceus* impacts cultivation. This study examined the effect of SPG addition at 0%, 10%, 20% and 30% of the substrate mixture by mass. However, all bags were contaminated and no *H. erincaceus* grew. This likely happened because thermogenic mycelial growth raised the substrate temperature past the optimal growth range, the rye berry inoculation mix was not allowed to grow long enough, and the substrate bags were not completely sterilized during autoclaving. These led to sub-optimal growth of *H. erinaceus*, allowing for it to be out-competed and unable to grow in the substrate bags. Following studies will work to reduce the thermogenic impact on substrate temperature, improve sterilization practices and increase the inoculation growth period.

1. Introduction

Hericium erinaceus (lion's mane) is a saprotrophic fungi that grows in Europe and the southern United States and is heavily cultivated in Asia[1]. In addition to being cultivated for consumption, recent studies have shown that it has medicinal properties. Polysaccharides derived from *H. erinaceus* potentially provide antitumor properties by interacting with various cell receptors[1]. Specifically, the lectin of *H. erinaceus* has shown anti-proliferation abilities in regard to certain breast cancer and hepatoma cells[2]. *Hericium erinaceus* were shown to stimulate macrophages, which protect against tumor cells and infected somatic cells. Additionally, the Chinese Food and Drug Administration has approved numerous medicines consisting solely of *H. erinaceus*[1]. Efficient and economic cultivation of *H. erinaceus* will help lower research and drug production costs.

In cultivation, *H. erinaceus* has been shown to have the highest growth rate on hardwood sawdust supplemented with wheat bran[3]. Additionally, studies have shown that addition of various agricultural and food waste products, like wheat straw, rice straw and sawdust, can supplement the growing process. The addidition of waste products allows for cheaper substrate production and a reduction of waste streams by using agricultural waste[4].

Spent coffee grounds (SCG) are a prominent example of an abundant waste which can supplement current substrates for *H. erinaceus* cultivation. SCG are plentiful, as coffee production passed 9.3 billion kilograms in 2016[5]. SCG are commonly deposited at landfills as they contain compounds detrimental to the composting process[5]. The utilization of SCG for *H. erinaceus* cultivation will improve waste management, reduce landfill use, and lower *H. erinaceus* production cost. SCG have been recommended to supplement *H. erinaceus* substrate[6]. However, no studies have examined the effects of different levels of SCG supplement on *H. erinaceus* cultivation.

This study investigated the effect of SCG supplementation to the substrate of *H. erinaceus* in order to find the most efficient use of SCG. SCG was substituted at 0%,10%, 20%, and 30% of total sawdust mass. The substrate lacking SCG will act as a control, allowing us to test the SCG supplementation to best known practices[6]. It is hypothesized that biological efficiency, which is the weight of the harvest divided by the dry weight of the substrate, will increase with the SGC percentage.

2. Methods

A liquid inoculant culture of 40 g of honey and one liter of water was mixed and sterilized in an autoclave for 45 minutes on the liquid cycle. *Hericium erinaceus* (strain Herin1) was purchased from Asheville Fungi (75 Thompson St, Asheville, NC 28803) and was then added into the liquid inoculant. Twenty to thirty ml of the liquid inoculant was poured into polypropylene bags with 200 g rye grain, 220 ml water, and 1 gram gypsum and left to grow for 7-12 days at 24 °C +/- 2° in one of two growth chambers (E-8 Reach-in Chamber, Conviron Environments Ltd. Winnipeg, Manitoba, Canada). A control group of 0.5 kg of substrate mixture containing 77% hardwood saw dust, 18% wheat bran, 3% grain inoculant, 1% calcium carbonate and 1% sucrose was created. Three treatment levels replacing 10%, 20% and 30% of the sawdust with SCG were created. Each treatment was replicated six times. Substrate mixtures for all treatments were added into polypropylene cultivation bags with gas exchange patches. All bags were autoclaved for a 45 minute liquid cycle at 121° C.

For each bag 0.93 kilograms of sterilized water was added to get a moisture content of 62%. After the substrate was mixed, placed into polypropylene bags, and autoclaved, the bags were inoculated with the spawn substrate mix. Bags were stored at 22°C ± 2° and 80% humidity for 16 days[7]. Once spawn run occurred the bags were moved to 12°C ± 2° growth chamber with 8 hours of artificial sunlight to stimulate primordial formation for 3-5 days. After this stimulation period, the growth chamber was adjusted to 20 °C ± 2° and three 10 cm long slits were cut after colonization to allow fruiting bodies to grow until spines reach five mm in length[6,7].

3. Results

The 24 bags of all treatment failed to produce the distinctive toothed white fruiting bodies of *Hericium erinaceus* fruiting bodies. 23 of the 24 bags showed signs of fungi distinct from *H. erinaceus (*Figure 1) while one bag fruited an unknown fungal species (Figure 2). White mycelium growth without contamination was observed in one bag, but that bag failed to produce any *H. erinaceus.*

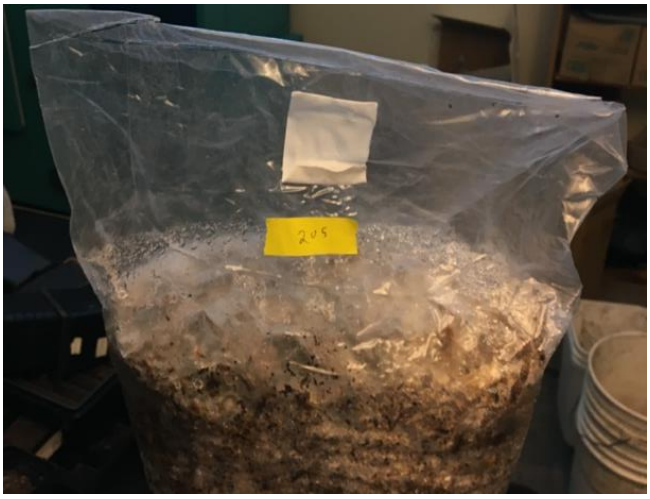

Figure 1. A substrate bag

One of the contaminated substrate bag showing fungal contamination distinct from the expected *Hericium erinaceus* mycelial growth.

Figure 2.Black fungal growth

One of the contaminated substrate bags, showing fruiting black fungal growth emerging from the openings in the bag.

4. Discussion

The lack of *H. erinaceus* fruiting indicates improper sterilization and experimental methods. There are a number of reasons the inoculated bags did not produce *H. erinaceus* fruiting bodies. First, incomplete sterilization of the substrate bags may have occurred. According to Stamets[6], the central core of the substrate bags is the slowest to heat. The substrate bags were autoclaved in a bin that pressed the side of the bags together. This increased the distance from the central core to the surrounding heated autoclave and the chance that the entire bag was not properly sterilized. Additionally, hardwoods, as used in this experiment, have been shown to have higher thermal inertia compared to lower density woods, indicating a longer autoclave period may have been necessary[6]. The incompletely sterilized bag allowed for organisms in the substrate bag to survive, grow and consume the nutrients prior to the substrate inoculant being added.

The spawn run of the *H. erinaceus* did not had enough time to grow before being stimulated for fruiting. Ideally, the spawn run should have a dense, distinct mycorrhizae network. The dense network increase the likelihood that *H. erinaceus* would outcompete any other organisms in the substrate bag. By outcompeting other organisms that contaminated the substrate for nutrients *H. erinaceus* would be able to fruit even under non-sterile conditions.

After inoculation a marked temperature increase, known as thermogenesis, occurs. If this raises the internal temperature past 38 °C, then most fungi cannot grow, and black pin molds and bacteria have been shown to propagate[6]. The black pin molds and bacteria colonize the substrate bag, consume nutrients and outcompete other organisms, not allowing other organisms to grow once the temperature returns to pre-thermogenesis levels[6]. Additionally, raising the temperature beyond the ideal growing conditions may reduce the growth rate of *H. erinaceus.* The specific thermogenic effect for fungal species is thought to be dependent on the rate of metabolic activity and the current temperature[8]. To lessen the thermogenic effect substrate bags are recommended to be placed on wire racks with ample space between bags[6]. This was not possible in the two growth chambers used. Additionally, using an intermediary inoculant substrate between the rye berries and substrate mix has been shown to reduce thermogenesis[6].

The experiment would have benefited from better sterilization practices and a longer spawn run time to increase the likelihood of *H. erinaceus* fruiting. The longer spawn run would have allowed *H. erinaceus* to establish a dense mycorrhizal network and increase its chance to outcompete potential contaminants in the substrate mixture. The dense mycorrhizal network allows for quick colonization, resource consumption and growth throughout the substrate bag.

Experimenter error could have caused external contamination after the initial sterilization at the inoculation phase for the liquid culture, wheat bran, or substrate mix. Contamination at any stage of the experiment would impact all further inoculations. A contamination likely occurred at the liquid culture or wheat bran inoculant phase, as the vast majority of bags showed infection. Future experiments would benefit from a number of changes. First, using sawdust as an intermediary inoculation substrate would reduce fungal induced thermogenesis in the growth substrate. Second, spacing the substrate bags out in the growth chamber, allowing for air between bags to reduce the temperature, would reduce the effect of thermogenesis. If thermogenesis no longer raises the temperate into the favorable environmental conditions for black pin mold and bacterial growth, *H. erinaceus* increases its chances outcompete them. Third, autoclaving substrate bags with space between them reduces the distance to the central core increasing sterilization success. Lastly, reducing the mass of substrate bags would also decrease the distance to the central core improving sterility.

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6. References

1. He X, Wang X, Fang J, Chang Y, Ning N, Guo H, Huang L, Huang X, Zhao Z. Structures, biological activities, and industrial applications of the polysaccharides from *Hericium erinaceus* (Lion's Mane) mushroom: A review. International Journal of Biological Macromolecules. 2017;97:228–237. doi:10.1016/j.ijbiomac.2017.01.040

2. Li Y, Zhang G, Ng TB, Wang H. A novel lectin with antiproliferative and HIV-1 reverse transcriptase inhibitory activities from dried fruiting bodies of the monkey head mushroom . Journal of Biomedicine and Biotechnology. 2010;2010. doi:10.1155/2010/716515

3. Siwulski M, Sobieralski K. Influence of some growing substrate additives on the *Hericium erinaceus* (Bull. Fr.) Pers. yield. Sodinink Darzinink. 2005;24(3):250–253.

4. Hassan FR. Cultivation of the Monkey Head Mush room (*Hericium erinaceus*) in Egypt. Journal of Applied Sciences Research,. 2007;3(10):1229–1233.

5. McNutt J, He Q (Sophia). Spent coffee grounds: A review on current utilization. Journal of Industrial and Engineering Chemistry. 2018;71:78–88. doi:10.1016/J.JIEC.2018.11.054

6. Stamets P. Growing gourmet and medicinal mushrooms. Third ed. Berkeley, CA: Ten Speed Press; 2000.

7. Atila F, Tuzel Y, Fernández JA, Cano AF, Sen F. The effect of some agro– industrial wastes on yield, nutritional characteristics and antioxidant activities of *Hericium erinaceus* isolates. Scientia Horticulturae. 2018;238(May):246–254. doi:10.1016/j.scienta.2018.04.049

8. Li Y, Wadsö L, Larsson L, Bjurman J. 2007. Correlating two methods of quantifying fungal activity: Heat production by isothermal calorimetry and ergosterol amount by gas chromatography-tandem mass spectrometry. Thermochim Acta. 458(1–2):77–83. doi:10.1016/j.tca.2007.01.005.